SEAL

MINISTRY OF PRODUCTIVE ACTIVITIES
GENERAL DIRECTORATE FOR PRODUCTION DEVELOPMENT AND COMPETITIVITY
Italian Patent and Trademark Office
OFFICE G2

*******

Authentication of copy of documents concerning the Patent application for INDUSTRIAL INVENTION No. MI2002A000189

It is hereby certified that the attached copy is the true copy of the original documents filed with the above mentioned patent application whose data are shown in the enclosed filing certificate.

Rome,

Director of the Division
(signature)

SEAL

FORM A
DUTY STAMP

TO THE MINISTRY OF INDUSTRY COMMERCE AND HANDICRAFT
Italian Patent and Trademark Office - ROME
Patent Application for Industrial Invention, filing of reserves, advanced opening to public inspection

A. Applicant
1) Name GIULIANI S.P.A. (Joint Stock Company)
Residence MILANO code 00752450155
2) Name
Residence code

B. APPLICANT'S REPRESENTATIVE BEFORE IPTO
Surname, name Dr. Romano Appoloni et al Fiscal code
Name of the corresp. office MARCHI & PARTNERS S.R.L.
Address Via Pirelli 19 City Milan code 20124 prov MI

C. ELECTED DOMICILE OF THE ADDRESSEE see above
Address No. city code prov

D. TITLE proposed class, (sec./cl./ucl.) group/subgroup

COMPOSITION FOR PHARMACEUTICAL OR DIETETIC USE FOR COMBATING HAIR LOSS

ADVANCED OPENING TO PUBLIC INSPECTION yes___ no _X
in presence of amendment request: date no. of ref.:

E. NAMED INVENTORS

| surname, name | surname, name |
|---|---|
| 1) RINALDI Fabio | 3) GATTI Valter |
| 2) SORBELLINI Elisabetta | 4) MARCHIORETTO Danila Ingrid |

F. PRIORITY

| Country or Exhibition | Type of Priority | Appln. No. | Appln. date | Encl(yes/res) |
|---|---|---|---|---|
| 1) | | | | |
| 2) | | | | |

G. CENTRE FOR COLLECTING MICROORGANISMS'CULTURES, denomination

H. SPECIAL NOTES

| ENCLOSED DOCUMENTS No. Doc. | | RESERVES DISSOLUTION | |
|---|---|---|---|
| Doc.1) 2 prov. no. sheets 17 | abstract with main drawing, spec. and claims (compulsory 1 copy) | Date | Filing. No. |
| Doc.2) 2 prov. No. Draw. 03 | drawings (compulsory if cited in the description, 1 copy) | | |
| Doc.3) 1 res. | power of attorney or reference to general power of attorney | | |
| Doc.4) 0 res. | designation of inventor | | |
| Doc.5) 0 res. | priority doc. with Italian transl | Comparison single prio. | |
| Doc.6) 0 res. | authorisation or assignment deed | | |
| Doc.7) 0 res. | complete name of the applicant | | |

8) PAYMENT RECEIPT OF EURO 118,79 = compulsory

filled in on 31.01.2002 The applicant's signature Romano Appoloni follows yes/no NO
We require a certified copy of the present deed yes/no NO

---

PROVINCIAL OFFICE OF INDUSTRY COMMERCE HANDICRAFT OF MILAN code 15

FILING CERTIFICATE Application no. Reg. A

The year 2001, the day of the month of

The above mentioned applicant(s) has(have) presented to me undersigned the present application consisting of no. 00 additional sheets for the grant of the above patent.

I. NOTES OF THE RECORDING OFFICER

THE DEPOSITER (signature)

THE RECORDING OFFICER (signature)

FORM A

ABSTRACT OF THE INVENTION TOGETHER WITH MAIN DRAWING, DESCRIPTION AND CLAIM

Application No. Reg.A Filing date
Patent No. Date of grant

D. TITLE

COMPOSITION FOR PHARMACEUTICAL OR DIETETIC USE FOR COMBATING HAIR LOSS

L. ABSTRACT

The present invention relates to a novel use of the polyamine known as spermidine, i.e. N-(3-aminopropyl)trtramethylenediamine, as an active principle in the preparation of a composition for pharmaceutical or dietetic use in man for combating hair loss

M. DRAWING

Fig. 3

Description of the Patent Application for the industrial invention entitled:

# COMPOSITION FOR PHARMACEUTICAL OR DIETETIC USE FOR COMBATING HAIR LOSS

Applicant: GIULIANI S.p.A.

* * * * *

The present invention relates to a novel use of the polyamine known as spermidine, i.e. N-(3-aminopropyl)tetramethylenediamine.

It is known in the literature that compounds belonging to the class of aliphatic polyamines play a deciding role in controlling the biological mechanisms of growth, division and differentiation of cells and proliferation of animal tissues.

The polyamines in question essentially comprise the compounds putrescine, spermine and spermidine. The latter compound, i.e. N-(3-aminopropyl)tetramethylenediamine, owes its name to the fact that it was first discovered in human sperm. In reality, it is present in virtually all the bodily fluids (blood, saliva, tears and milk). It was subsequently also found in many foods of both animal origin (meat, fish, eggs, milk and cheese) and plant origin (fruit and vegetables). It is of particularly high concentration in human milk (on average about 600 micrograms in milk over 24 hours), where it plays an important role for the newborn. Specifically, in the newborn, the mucosae of the digestive tract are not fully formed and spermidine, taken up with the milk, promotes the growth of the epithelium of the gastric and intestinal mucosa.

Spermidine is therefore an important factor in the growth and proliferation of cells.

According to the present invention, it has now been found, surprisingly, that a preparation containing spermidine, administered orally to man, results in a stimulation of the hair bulbs with consequent promotion of hair growth, in particular in the case of a pathological hair loss such as that known as telogenic defluvium, characterized by a state of suffering of the hair bulb, leading to an abnormal and excessive loss of hair.



One subject of the present invention is, thus, the use of

spermidine as an active principle in the preparation of a composition for pharmaceutical or dietetic use in man to combat pathological hair loss, in particular in the case of telogenic defluvium.

A subject of the present invention is also a composition for pharmaceutical or dietetic use to be administered to man to combat pathological hair loss, characterized in that it comprises spermidine as active principle.

To understand the characteristics and advantages of the invention more clearly, an experimental study from which they are derived will now be described in greater detail.

To this end, a few fundamental notions regarding the phases of growth of a hair should first be presented. The growth cycle of hair consists essentially of three phases, during which the hair follicle passes from periods of intense growth to periods of quiescence and then of involution. These three phases are: the anagenic phase, i.e. the phase of hair growth, during which there are a number of changes in the dermal papilla in which the cells undergo intense metabolic activity. The hairs grow 0.3-0.4 mm per day. The hairs are not all in the same growth phase, but rather they alternate. The anagenic phase lasts from 3 to 6 years.

The catagenic phase is the phase of involution lasting from 2 to 3 weeks, during which the hair follicle undergoes profound morphological and metabolic changes. The lower segment is lost, the length of the follicle is reduced by about a third, the bulb decreases in size, the melanocytes stop producing pigment and the papilla becomes atrophic: the hair falls out.

Finally, the telogenic phase is the resting phase, during which the hair follicle is completely inactive. The hair is inside the hair follicle, held by weak intercellular bonds that cause it to stay in the scalp until the start of the new anagenic phase and sometimes even for several

successive phases. The telogenic phase lasts from 2 to 4 months.

Every day about 50 hairs die and fall out, and, under normal conditions, are immediately replaced by new members, since the follicles have mutually synchronized life cycles such that the total volume of hairs remains virtually unchanged. In this way, total exchange of the hairs takes place every 2-6 months.

The concept of telogenic defluvium was first introduced by Kligman in 1961. Before that time, it was difficult to distinguish the causes of excessive hair loss (owing to metabolic disturbances, intoxication or infection) from the other more general forms of alopecia.

The diagnosis of telogenic defluvium is made by taking into consideration the growth phases of the hair. When, for various reasons, the anagenic and telogenic phases are altered (and this may take place in both senses: either they are excessively faster or excessively slower than the norm), this results in the phenomenon of telogenic defluvium, which is distinguished by excessive hair loss and by profound morphological alterations in the hair.

The factors that can lead to an imbalance in the hair cycles, with consequent onset of telogenic defluvium, may be: particular physiological conditions (pregnancy), prolonged states of stress and anxiety, use of certain drugs such as, for example, bromocryptine, cimetidine, levodopa, etretinate, lithium, pyridostigmine, propanolol and anti-thyroid drugs, non-balanced diets and deficiencies in vitamins and minerals.

The morphological alterations in the hair during telogenic defluvium may be a microscopically visible destructuring of the shaft, with a consequent reduced mechanical tensile strength and reduced elasticity; alterations in the trichogram; mineral deficiencies, or histological alterations in the hair bulb.

## CLINICAL STUDY

This controlled, randomized double-blind study was performed according to the present invention by means of the protocols below.

Sixty volunteers of both sexes and ranging between 18 and 60 years old were divided into three groups of 20 individuals each, all having the same level of pathology, i.e. telogenic defluvium existing for at least 2 months.

Some of these individuals were treated for 60 days with one capsule a day of a composition of the invention, according to the following scheme:

Group 1: 20 individuals treated with a composition of the invention containing spermidine alone (0.50 mg per capsule).

Group 2: 20 individuals treated with a composition of the invention according to Example 1 described later (spermidine 0.50 mg per capsule).

Group 3: 20 individuals treated with a placebo, in capsules.

The parameters evaluated were the following:

A) General dermatological visit.

B) Microscopic evaluation of the shaft of the hairs (diameter and possible structural changes in the hair).

C) Trichogram, i.e. evaluation of the bulbs in the anagenic (growth) phase, catagenic (involutive stasis) phase, telogenic (pathological precocious hair loss) phase and exogenic (physiological loss of hairs since they are replaced with new hairs).

D) Haematochemical analysis.

E) Pull test (mechanical tensile strength of the hair).

F) Wash test (count of the hairs lost after washing with shampoo).

G) Possible side effects.

These parameters were evaluated at time $T_0$ (before the start of the treatment); at time $T_1$ (at the end of the 60 days of treatment); and

finally at time $T_2$ (30 days after stopping the administration).

The results were as follows:

A) The dermatological visit revealed an appreciable and significant reduction in the hair loss and an improvement in the structure of the shaft in the groups of patients 1 and 2 compared with the placebo group 3.

B) Microscopic evaluation of the shaft

The diameter of the hair shaft increases quite substantially in groups 1 and 2, whereas it remains virtually unchanged in the placebo group 3.

C) The trichogram is the parameter that, together with the wash test, gave the most interesting results. In this regard, reference is made to the diagrams of Figures 1 and 2 in the attached drawings.

These show the trichogram of the anagenic and telogenic phases at times $T_0$ (before the start of the treatment); at time $T_1$ (at the end of the 60 days of treatment); and finally at time $T_2$ (30 days after stopping the administration) for the individuals of the three groups, 1 (grey column), 2 (black column) and 3 (pale column).

The percentage of hairs in the anagenic phase (Fig. 1) and in the telogenic phase (Fig. 2) for the treated individuals belonging to the three groups under consideration is shown on the y-axis, and the said times T are shown on the x-axis, under which are tabulated the said percentages found.

Specifically, the microscopic analysis of the state of the bulb reveals that the number of bulbs in the anagenic phase increases significantly in Groups 1 and 2 and, in parallel, in the same Groups, the telogenic phase decreases significantly. In contrast, in the placebo group 3, the anagenic and telogenic phases are not significantly changed.

In particular, a 17.2% increase in the anagenic phase at $T_2$ relative

to $T_0$ (with an 8.1% increase at $T_1$) was found in Group 1.

A 20.2% increase in the anagenic phase at $T_2$ relative to $T_0$ (with an 8.12% increase at $T_1$) was found in Group 2.

A 7.79% increase in the anagenic phase at $T_2$ relative to $T_0$ (with a 2.7% increase at $T_1$) was found in Group 3. The change of about 7.79% in the anagenic phase from $T_0$ to $T_2$ in the placebo group is comparable to the cyclic changes that take place within the hair bulbs.

In parallel, the telogenic phase decreased by:

6.76% at $T_2$ (9.1% at $T_1$) in Group 1

27.7% at $T_2$ (9.6% at $T_1$) in Group 2

4.16% at $T_2$ in Group 3, for which, however, an increase in the telogenic phase (of about 1.88%) is actually found at $T_1$.

D) The haematochemical analyses gave values within the norm for all the Groups 1, 2 and 3.

E) Pull test:

The mechanical tensile strength of the hair increased significantly in Groups 1 and 2, whereas it remained virtually unchanged in the placebo Group 3.

F) Wash test. This test makes it possible not only to quantify the number of hairs lost after shampooing, but also, by means of a suitable microscopic analysis, to evaluate the phase of the cycle in which the bulb was found when the hair fell out: pathological loss (telogenic) or phase of physiological exchange (exogenic).

The test results are given in the graph of Figure 3 in the attached drawings. In this graph, the number of hairs lost during washing for the individuals of the three groups mentioned above is given on the y-axis, and the said times T [$T_0$ (before starting the treatment); $T_1$ (at the end of the 60 days of treatment); $T_2$ (30 days after stopping the administration)] are given on the x-axis, under which are tabulated the values found.

As may be seen, the number of hairs lost in the wash test

decreases significantly for the treatment process in Groups 1 and 2 (solid lines), whereas it remains unchanged in the placebo Group 3 (dashed line in the graph).

In addition, by analysing the bulbs of the lost hairs, the following important observation was made. In Group 3 treated with placebo, more than 90% of the lost hairs were in the telogenic phase (pathological loss) and only 3% were in the exogenic phase (physiological loss).

In Groups 1 and 2 this ratio changes, since the hairs in the exogenic phase are 33% in Group 1 and 46% in Group 2, with a consequent reduction in the hairs in the telogenic phase, which was found to be 63% in Group 1 and 52% in Group 2.

Thus, in Groups 1 and 2, among the lost hairs, there was a significant decrease in the percentage of hairs in the telogenic phase (pathological loss) and a proportionate increase in the percentage of hairs in the exogenic phase (physiological loss by exchange).

G) The side effects were mild and all disappeared as the treatment continued, taking care to take the capsule during the main meal, at $T_1$.

According to the present invention, it was thus experimentally found that the oral administration to man of a composition containing spermidine, preferably combined with other components such as methionine, bioflavonoids, vitamins and mineral salts, is capable of slowing down and stopping excessive hair loss in the case of telogenic defluvium, and simultaneously of improving the strength and general health of the hair.

The pull test demonstrated that spermidine, either in unmodified form or combined with other micro-nutrients, increases the mechanical tensile strength of the hair.

The trichogram and the wash test made it possible to demonstrate large variations arising in the hair bulb following treatments with

spermidine in unmodified form or combined with other active components. Not only is the number of hairs lost after washing substantially reduced, but also, among those lost, the number in the telogenic phase (pathological loss) is substantially decreased when compared with the number in the exogenic phase (loss by physiological exchange). Thus, the treatment with spermidine in unmodified form or with spermidine combined with other micro-nutrients has substantially modified the cycle of the hair altered by the telogenic defluvium pathology, returning it to the normal values of physiological exchange.

For the use of spermidine according to the present invention, it is convenient to formulate it in compositions preferably for oral use, and preferably as a dietetic product. It may also be formulated in compositions for topical use on the scalp.

A number of examples, not intended to be limiting, of compositions according to the invention will now be described.

EXAMPLE 1

DIETETIC COMPOSITION FOR MAKING THE HAIR ROBUST AND REDUCING HAIR LOSS

Sealed rigid plant capsules

Each capsule contains:

| Active principles | | |
|---|---|---|
| Methionine | 300.00 | mg |
| Vitamin C | 90.00 | mg |
| Polyphenols from *Vitis vinifera* | 5.00 | mg |
| Vitamin E | 15.00 | mg |
| Calcium pantothenate | 9.00 | mg |
| Zinc (as amino acid chelate) | 7.50 | mg |
| Vitamin $B_6$ | 2.00 | mg |
| Copper (as amino acid chelate) | 1.25 | mg |

| | | | |
|---|---|---|---|
| | Spermidine | 0.50 | mg |
| | Folic acid | 0.15 | mg |
| | Biotin | 0.05 | mg |
| | Excipients | | |
| 5 | Hydroxypropylmethylcellulose | 110.00 | mg |
| | Talc | 21.00 | mg |
| | Magnesium stearate | 6.50 | mg |
| | Colloidal silica | 2.85 | mg |
| | Natural colorants | 2.50 | mg |

10 EXAMPLE 2

DIETETIC COMPOSITION FOR MAKING THE HAIR ROBUST AND REDUCING HAIR LOSS

Packets to be dissolved in water

Each packet contains:

| | | | |
|---|---|---|---|
| 15 | Active principles | | |
| | Methionine | 300.00 | mg |
| | Vitamin C | 90.00 | mg |
| | Polyphenols from *Vitis vinifera* | 20.00 | mg |
| | Vitamin E | 15.00 | mg |
| 20 | Calcium pantothenate | 9.00 | mg |
| | Zinc (as amino acid chelate) | 7.50 | mg |
| | Beta-carotene | 4.20 | mg |
| | Vitamin $B_6$ | 2.00 | mg |
| | Copper (as amino acid chelate) | 1.25 | mg |
| 25 | Spermidine | 0.50 | mg |
| | Folic acid | 0.30 | mg |
| | Biotin | 0.15 | mg |
| | Excipients | | |
| | Maltodextrin | 2 000.00 | mg |
| 30 | Sodium citrate | 350.00 | mg |

| | | | |
|---|---|---|---|
| | Citric acid monohydrate | 200.00 | mg |
| | Flavourings | 160.00 | mg |
| | Colloidal silica | 65.00 | mg |
| | Aspartame | 30.00 | mg |
| 5 | Acesulfame K | 7.00 | mg |
| | Natural colorants | 3.50 | mg |

EXAMPLE 3

COSMETIC ATTACK LOTION (initial treatment) FOR MAKING THE HAIR ROBUST AND REDUCING HAIR LOSS

10 In ampules

Each ampule of 10 ml of solution contains:

Active principles

| | | | |
|---|---|---|---|
| | Spermidine | 2 | mg |
| | Catechin and quercetin complex | 40 | mg |
| 15 | Methylsulphonylmethane | 400 | mg |
| | Azeoglycine (potassium azeloyl diglycinate) | 300 | mg |
| | Sunflower oil and rosemary oil | 5 | mg |
| | Menthyl lactate | 25 | mg |
| | Calcium pantothenate | 16 | mg |
| 20 | Biotin | 0.15 | mg |

Excipients

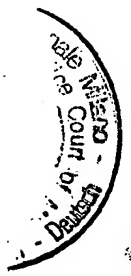
| | | | |
|---|---|---|---|
| | Ethyl alcohol | 4.0 | ml |
| | Fragrance | 5.0 | mg |
| | Natural colorants | 0.2 | mg |
| 25 | Purified water qs | 10 | ml |

EXAMPLE 4

COSMETIC MAINTENANCE LOTION (continuation of treatment) FOR MAKING THE HAIR ROBUST AND REDUCING HAIR LOSS

In bottles

30 100 ml of solution contain:



| | | | |
|---|---|---|---|
| | Active principles | | |
| | Spermidine | | 5 mg |
| | Catechin and quercetin complex | | 200 mg |
| | Methylsulphonylmethane | | 2 000 mg |
| 5 | Azeoglycine (potassium azeloyl diglycinate) | | 3 000 mg |
| | Sunflower oil and rosemary oil | | 50 mg |
| | Menthyl lactate | | 250 mg |
| | Calcium pantothenate | | 80 mg |
| | Biotin | | 1.5 mg |
| 10 | Excipients | | |
| | Ethyl alcohol | | 35 ml |
| | Natural colorants | | 900 mg |
| | Fragrance | | 50 mg |
| | Purified water | qs | 100 ml |

15 EXAMPLE 5

COSMETIC BALM FOR MAKING THE HAIR ROBUST AND REDUCING HAIR LOSS

In bottles

100 ml of balm contain:

| | | |
|---|---|---|
| 20 | Active principles | |
| | Spermidine | 10 mg |
| | Catechin and quercetin complex | 400 mg |
| | Methylsulphonylmethane | 4 000 mg |
| | Azeoglycine (potassium azeloyl diglycinate) | 3 000 mg |
| 25 | Sunflower oil and rosemary oil | 50 mg |
| | Menthyl lactate | 250 mg |
| | Calcium pantothenate | 80 mg |
| | Biotin | 1.5 mg |
| | Excipients | |
| 30 | Cetearyl alcohol | 5 000 mg |

| | | | |
|---|---|---|---|
| | PEG-15 cocopolyamine | | 5 000 mg |
| | Oat protein hydrolysate | | 3 000 mg |
| | Glycerol | | 3 000 mg |
| | Cetyl alcohol | | 2 000 mg |
| | Quaternium-52 | | 1 000 mg |
| | Phenoxyethanol | | 300 mg |
| | Methyl-ethyl-propyl para-oxybenzoates | | 200 mg |
| | Fragrance | | 500 mg |
| | Colorants | | 1 000 mg |
| 10 | Purified water | qs | 100 ml |

CLAIMS

1. Use of spermidine as an active principle in the preparation of a composition for pharmaceutical or dietetic use in man to combat hair loss.

2. Use of spermidine according to Claim 1, to combat hair loss in the case of the pathology known as telogenic defluvium.

3. Use of spermidine according to Claim 2, to reduce the telogenic phase in the growth cycle of the hair.

4. Use of spermidine according to Claim 1, to make the hair robust.

5. Composition for pharmaceutical or dietetic use to be administered to man to combat hair loss, characterized in that it comprises spermidine as active principle.

6. Composition according to Claim 5, characterized in that it comprises methionine, vitamin C, polyphenols, vitamin E, calcium pantothenate, zinc (as amino acid chelate), vitamin $B_6$, copper (as amino acid chelate), folic acid and biotin.

7. Composition according to Claim 6, characterized in that it comprises:

| | | |
|---|---|---|
| Methionine | 300.00 | mg |
| Vitamin C | 90.00 | mg |
| Polyphenols from *Vitis vinifera* | 5.00 | mg |
| Vitamin E | 15.00 | mg |
| Calcium pantothenate | 9.00 | mg |
| Zinc (as amino acid chelate) | 7.50 | mg |
| Vitamin $B_6$ | 2.00 | mg |
| Copper (as amino acid chelate) | 1.25 | mg |
| Spermidine | 0.50 | mg |
| Folic acid | 0.15 | mg |
| Biotin | 0.05 | mg |

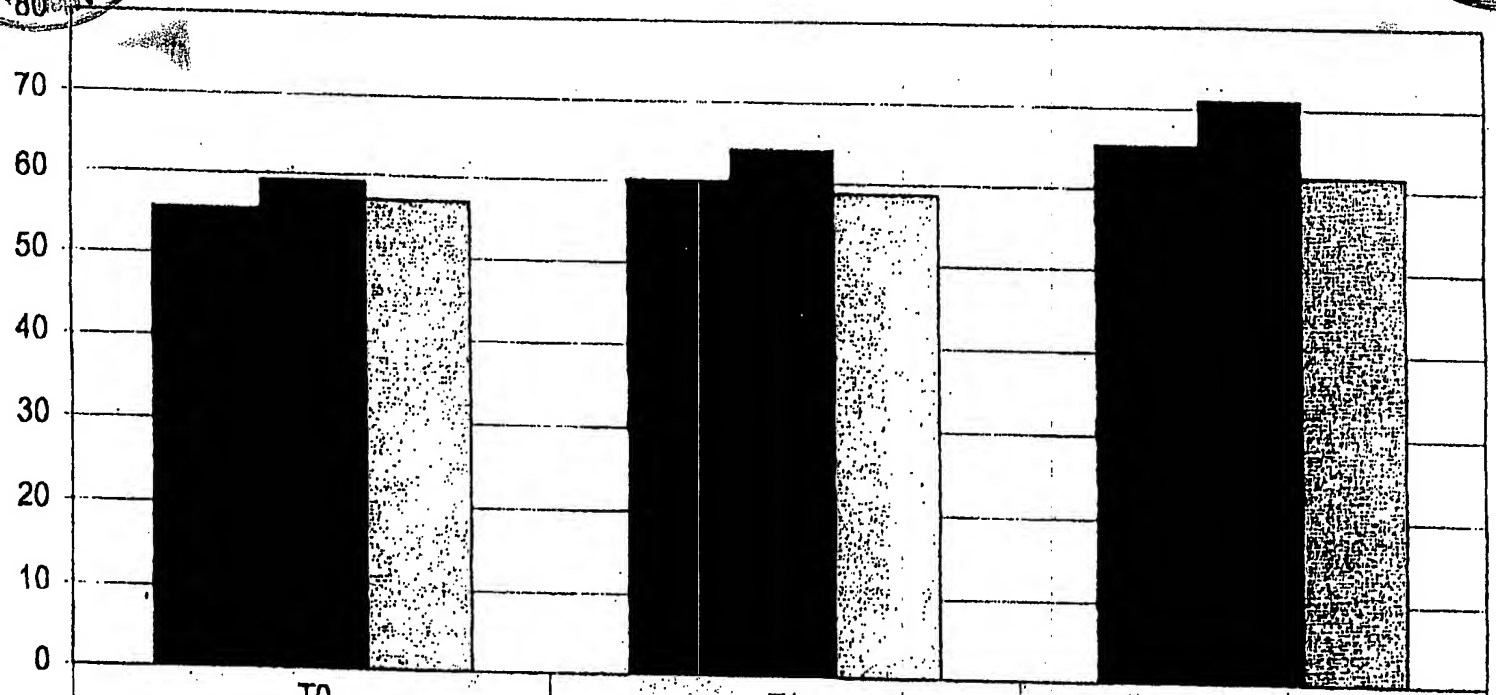
8. Composition according to Claim 5, characterized in that it is

suitable for oral administration.

9. Composition according to Claim 5, characterized in that it is suitable for topical administration, as a lotion or a balm.

10. Composition according to Claim 5, characterized in that it is a product for dietetic use to make the hair robust and to reduce hair loss.

Dr. Romano Appoloni




| | T0 | T1 | T2 |
|---|---|---|---|
| 1 | 55.6 | 60.15 | 65.2 |
| 2 | 59.1 | 63.9 | 71.05 |
| 3 | 57.1 | 58.65 | 61.55 |

Fig. 1

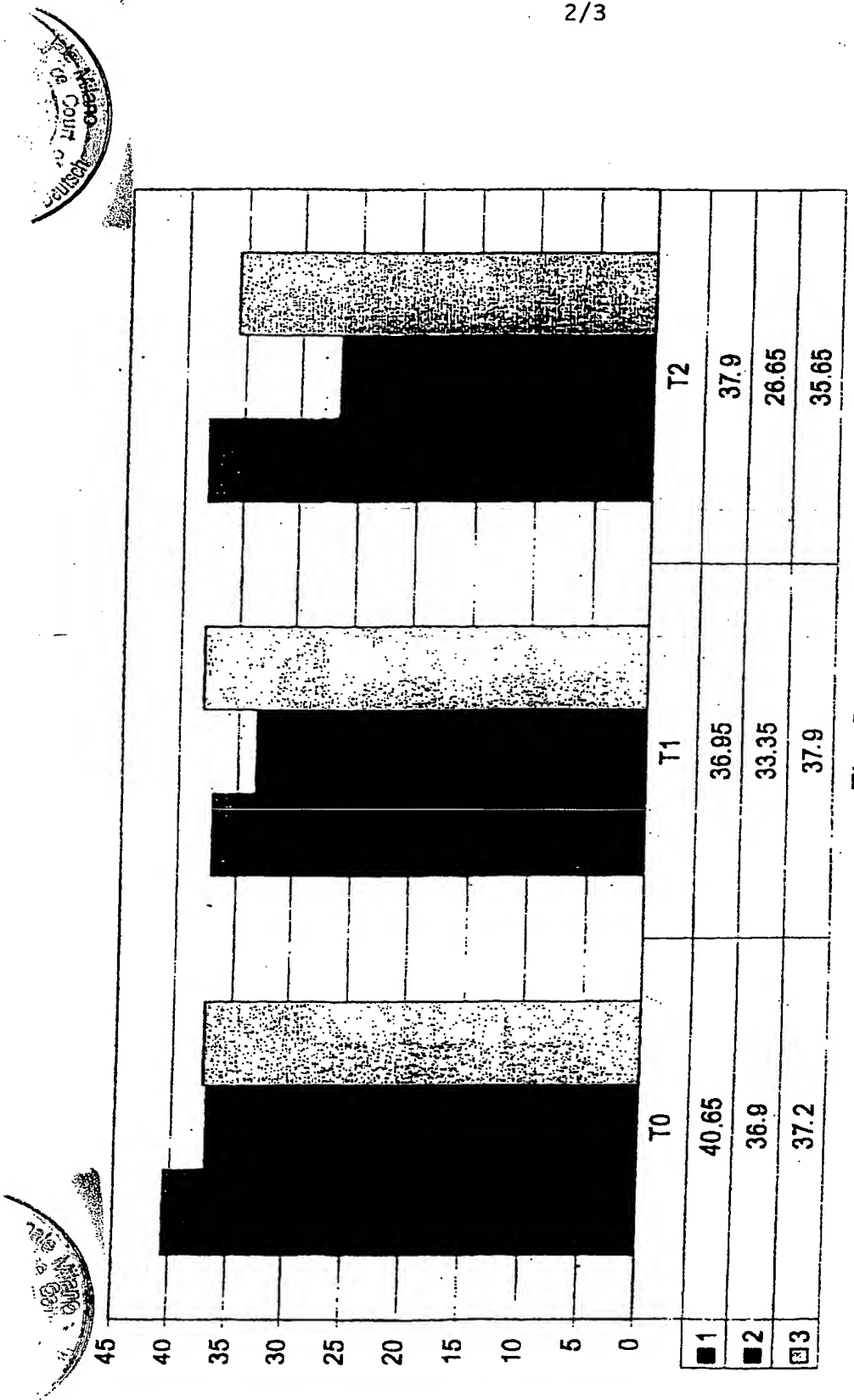

45
40
35
30
25
20
15
10
5
0
T0
T1
T2
1
2
3
40.65
36.9
37.2
36.95
33.35
37.9
37.9
26.65
35.65

Fig. 2

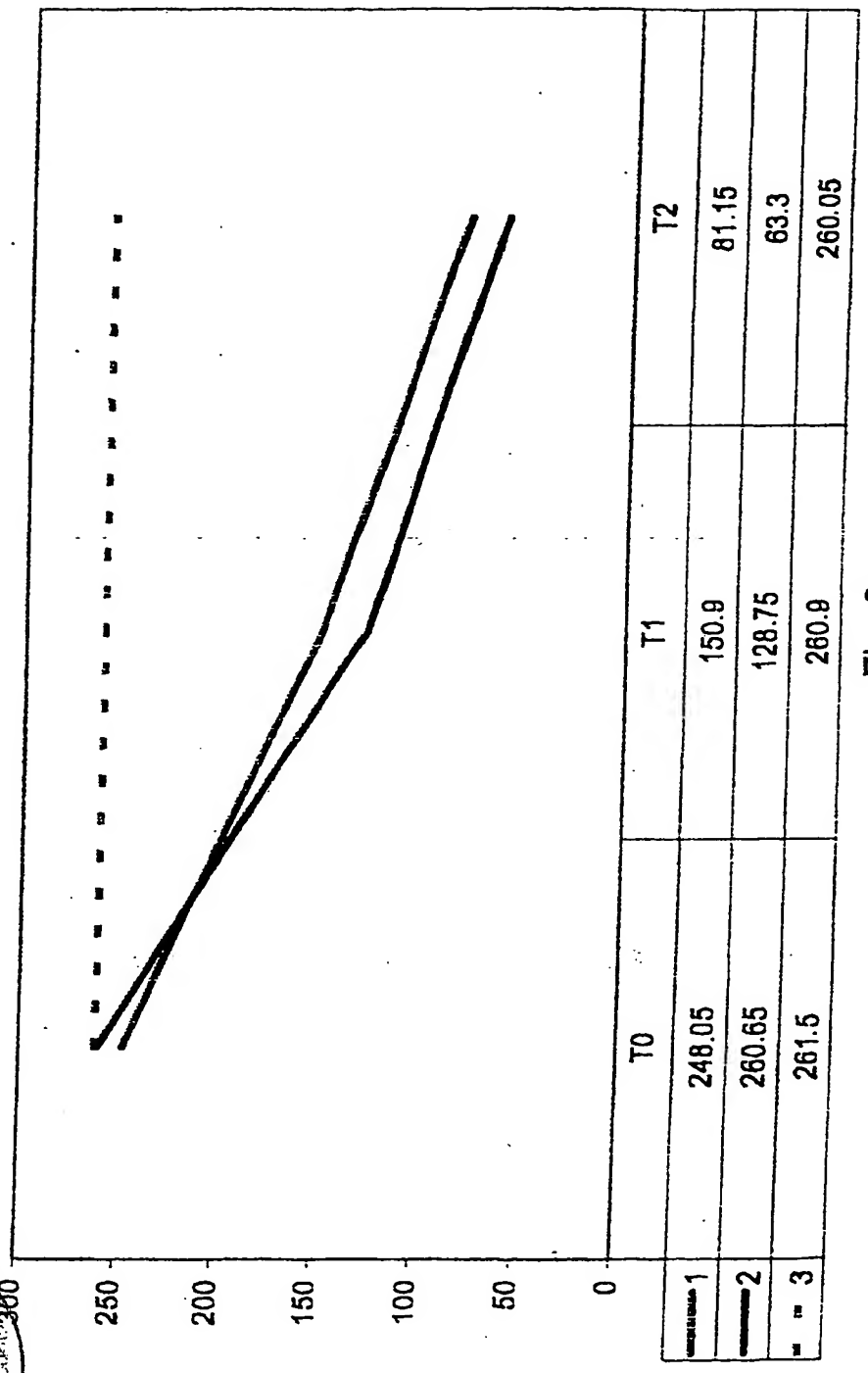

300
250
200
150
100
50
0
T0
T1
T2
1
248.05
150.9
81.15
2
260.65
128.75
63.3
3
261.5
260.8
260.05

Fig. 3

## DECLARATION UNDER 37 CFR 1.68

I, Giovanna Luisa Sarolo, declare

That I reside at Via Podgora 6, Milan, Italy;

That I am familiar with the Italian and English languages;

That I am a Sworn Translator, appointed by the Court of Milan, Italy;

That I have prepared the attached translation of the Italian Patent Application No. **MI2002A000189** filed on **01 February 2002** with the title: "Composition for pharmaceutical or dietetic use for combating hair loss", said Italian language document being already filed at WIPO during the PCT procedure.

That the attached translation is complete and accurate and fairly reflects the meaning and content of said Italian language document.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Giovanna Luisa SAROLO

Milan, ITALY,

8 th Mar. 2005

Traduttore Tribunale Milano - Consulente Translator Appointed by Justice Court of Milan - Français - English - Deutsch
GIOVANNA LUISA SAROLO
MILANO
Via Podgora, 6
ITALIA